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Physical and oxidative stability of fish oil nanoemulsions produced by spontaneous emulsification: Effect of surfactant concentration and particle size

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ABSTRACT

Nanoemulsion-based delivery systems offer many potential benefits for incorporating omega-3 oils into foods and beverages. Nanoemulsions are gaining popularity because of their ease of preparation, small particle size, relatively high stability, and production of optically transparent emulsions. In this study, spontaneous emulsification, a low-energy method, was used to fabricate fish oil nanoemulsions. The influence of surfactant-to-oil-ratio on particle size and physical stability was evaluated. Optically transparent nanoemulsions were formed and maintained physical stability at 37 °C for 14 days. Furthermore, the effect of particle size and surfactant concentration on oxidative stability of these nanoemulsions was compared to emulsions produced by microfluidizer, a high-energy method. These nanoemulsions had similar oxidative stabilities at 55 °C for 14 days. These results demonstrate that spontaneous emulsification can produce fish oil nanoemulsions that are physically stable and oxidize at similar rates as traditionally prepared nanoemulsions, and therefore may be suitable for fortifying clear food systems.

1. Introduction

Fish oil is an excellent source of long chain polyunsaturated fatty acids (PUFAs), such as eicosapentaenoic acid (EPA, 20:5 n-3) and docosahexaenoic acid (DHA, 22:6 n-3) (Maki et al., 2014). Consumption of adequate levels of fish oil has been shown to provide health benefits associated with brain development, inflammation, and cardiovascular disease (Kris-Etherton et al., 2009). In the United States, the 2010 Dietary Guidelines for Americans (USDA) recommends the consumption of 250 mg of EPA and DHA per day through the means of 8 oz (227 g) of a variety of seafood a week. Unfortunately, Americans are falling short of this recommendation with a current consumption of 3.5 oz (99 g) of seafood per week, mostly from sources low in omega-3 FAs (Kris-Etherton et al., 2009). This under-consumption of seafood may be attributed to taste, price, contamination concerns (such as heavy metals), and availability (Glanz et al., 1998; Kennedy et al., 2012; Racine and Deckelbaum, 2007). As a result, there is a need to develop alternative sources for omega-3 FAs in consumer's diets. Emulsion-based delivery systems are particularly suitable for incorporating fish oils into functional food products.

Nanoemulsions, a class of emulsion-based delivery systems, have been of particular interest lately because of their simple fabrication, high physical stability, and high bioavailability (McClements, 2011). By definition, nanoemulsions have a mean droplet radii below 100 nm, and may become optically transparent at sufficiently small particle sizes (Mason et al., 2006; Tadros et al., 2004). In contrast to microemulsions, which are thermodynamically stable systems, nanoemulsions are thermodynamically unstable systems but can be designed to be kinetically stable (Anton et al., 2008; Anton and Vandamme, 2011; McClements, 2012). Nanoemulsions can be fabricated by high or low-energy methods. High-energy methods use specialized mechanical devices to breakdown the droplets into very fine particles, such as microfluidizers (MF), high pressure valve homogenizers, or sonicators (McClements, 2011; McClements and Rao, 2011; Tadros et al., 2004). In contrast, low-energy methods are able to spontaneously form very fine droplets as a result of controlled changes in the environment or solution conditions (McClements and Rao, 2011; Solans and Sole, 2012). The interest in low-energy methods for certain applications is increasing because of their lower manufacturing costs, simple production methods, and ability to create smaller particle sizes than high-energy methods (McClements

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and Rao, 2011). Spontaneous emulsification (SE) is one of the simplest low-energy methods to implement since it only involves the addition of one phase into another phase with continuous stirring to spontaneously form a nanoemulsion (Anton and Vandamme, 2009; Saberi et al., 2013). Typically in SE, the organic phase consisting of oil and surfactant is added to the aqueous phase with mild stirring.

This study will focus on the potential of spontaneous emulsification to fabricate fish oil nanoemulsions that are suitable for application in clear beverages. As part of this study, the nanoemulsions created using this low-energy method will be compared to those produced using a high-energy method (MF) to highlight the advantages and limitations of these different approaches. Previous studies haves shown that microfluidization is a particularly efficient high-energy method of producing nanoemulsions containing small lipid droplets (Jafari et al., 2008, 2007, 2006). Both the physical and chemical stability of nanoemulsions are obstacles that must be addressed when producing foods fortified with omega-3 fatty acids (McClements et al., 2007), and so these issues will be evaluated in this research.

2. Materials and methods

2.1. Materials

Fish oil (FO) (Ropufa 30 n-3 food oil) was provided by DSM Nutritional Products Ltd. (Basal, Switzerland). The oil was composed of 101 mg of EPA/g of oil, 148 mg of DHA/g oil, and 312 mg of total n-3 PUFA/g of oil. Lemon oil (LO) was kindly donated by Citrus & Allied Essences (Lake Success, NY, USA). The supplier reported the chemical composition as determined by gas chromatography (Table 1). Non-ionic surfactant, polysorbate 80 (Tween 80), sodium benzoate, thiobarbituric acid (TBA), butylated hydroxytoluene (BHT), 1,1,3,3-tetraethoxypropane (TEP), barium chloride, iron (II) sulfate heptahydrate, hydrochloric acid, and cumene hydroperoxide were purchased from Sigma–Aldrich Co. (St. Lois, MO, USA). Citric acid, isooctane, 1,2-propanol, methanol, and butanol were purchased from Fisher Scientific (Waltham, MA, USA). Trichloroacetic acid (TCA) and ammonium thiocyanate were purchased from Acros Organics (Geel, Belgium). Ethanol was purchased from Pharmco-AAPER (Brookfield, CT, USA). All solvents and reagents were of analytical grade or higher. Double distilled water was used to prepare all solutions.

Table 1
Concentration of constituents in threefold (3×) lemon oil, provided by Citrus & Allied Essences (Lake Success, NY).

Constituent	Concentration in lemon oil (%)
α-Thujene	0.00
α-Pinene	0.70
Camphene	0.00
Sabinene	0.30
β-Pinene	4.90
Myrcene	0.80
Octanal	0.01
Limonene	63.00
α-Phellandrene	0.02
γ-Terpinene	14.00
Terpinolene	1.80
Linalool	0.60
Citronellal	0.30
α-Terpineol	0.30
Neral (citral B)	2.38
Geranial (citral A)	3.84
Neryl acetate	0.97
Geranyl acetate	0.60
(E)-caryophyllene	0.90
Trans-α-bergamotene	0.90
β-Bisabolene	1.40
Total (%)	97.72

2.2. Emulsion preparation

2.2.1. Low-energy method: Spontaneous emulsification

Nanoemulsions were prepared by spontaneous emulsification (SE) for physical and oxidation stability evaluation. The organic phase consisted of a mixture of fish oil (5 wt%) and lemon oil (5 wt%), which were stirred at 750 rpm for 15 min, and then stirred for an additional 30 min after adding non-ionic surfactant (2.5–20 wt% Tween 80). The aqueous phase was buffer (70–87.5 wt%) consisting of 0.8 wt% citric acid and 0.08 wt% sodium benzoate at pH 3.0, in order to simulate the aqueous phase of a beverage system. In this method, the organic phase was added to an aqueous phase using an automatic pipette (Ranin 10 mL E4 XLS, Mettler-Toledo International Inc., Columbus, OH, USA) while stirring at 500 rpm for 15 min.

2.2.2. High-energy method: Microfluidizer

Nanoemulsions were also prepared by microfluidization (MF) for oxidation stability evaluation. Fish oil (10 wt%) and lemon oil (10 wt%) were mixed for 15 min at 750 rpm. Buffer (78 wt%) was mixed with Tween 80 (2 wt%) for 30 min at 750 rpm. The two phases were added together and mixed for 2 min with a hand mixer (Bamix ESGE Ltd., Switzerland) to form a course emulsion. Samples were passed through a microfluidizer (M-110L, Microfluidics, Newton, MA) 3 times at 12,000 PSI.

2.2.3. Post-production alterations of emulsions

All emulsions were diluted to 1 wt% oil (0.5 wt% FO and 0.5 wt% LO) with buffer solution and then stirred for 5 min at 300 rpm. Finished emulsions were held in 50 mL disposable centrifuge polypropylene tubes (Fisher Scientific, Pittsburg, PA, USA). Each formulation was made in duplicate.

For the emulsions used in oxidation studies, additional surfactant was added during the dilution stage to evaluate the effect of surfactant and particle size on oxidation. For these emulsions, Tween 80 was mixed with the volume of buffer used for dilution at 750 rpm for 30 min. This solution was added to the emulsion to dilute to 1 wt% oil (0.5 wt% FO and 0.5 wt% LO) and stirred at 300 rpm for 5 min. Iron (100 μM) as $FeSO_4$ was also added to all emulsions used in the oxidation studies to accelerate the lipid oxidation reaction. Emulsions were observed using optical microscopy on day 0 and 14 of the oxidation experiments (Nikon Eclipse 80i, Nikon Instrument Inc., Melville, NY). Each formulation was made in duplicate.

2.3. Surfactant concentration

The effect of surfactant concentration in the SE nanoemulsions was evaluated by varying the surfactant-to-oil ratio (SOR) while keeping the total amount of oil (fish oil and lemon oil) constant (10 wt%).

$$\%\mathrm{SOR} = 100 \times m_s/m_o$$

where m_s is the mass of the surfactant and m_o is the total mass of the oil phase.

2.4. Particle size measurements

The particle size distribution (PSD) of all emulsions was measured by either dynamic (Zetasizer Nano ZS, Malvern Instruments, Malvern, UK) or static light scattering instruments (Mastersizer 2000, Malvern Instruments, Malvern, UK). Static light scattering was used to measure the size of the droplets in